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Thin-Layer Chromatography of Carbonyl Compounds
Separation of Dicarbonyl bis(2,4-Dinitrophenylhydrazones) into Classes

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INTRODUCTION

Although a number of methods have been described for class separation of monocarbonyl 2,4-dinitrophenylhydrazones by thin-layer chromatography (TLC) (*1*, *2*, *4*), no reports have appeared on the use of this method for the separation of the bis(2,4-dinitrophenylhydrazones) of dicarbonyls. This paper describes a method for the successful resolution into classes of the bis(2,4-dinitrophenylhydrazones) of vicinal dicarbonyls on plates coated with thin layers of a weakened magnesia-Celite mixture. The classes investigated were the 2,3-diketones, the α-keto aldehydes, and the dialdehyde, glyoxal. The technique as described below will readily permit the classification of as little as 4×10^{-5} μmole of a vicinal dicarbonyl bis(2,4-dinitrophenylhydrazone) in about 40 minutes.

MATERIALS AND METHODS

Seasorb 43 (adsorptive magnesia) obtained from the Fisher Scientific Co.,[1] Silver Spring, Maryland, is partially deactivated by heating it in a muffle furnace at 525° ± 25°C for 16 hours. Celite 545 (Johns-Manville Co.)[1] is dried at 100° for 24 hours.

Preparation of plates. The TLC equipment is identical to that described by Schwartz and Parks (*2*). The plates are prepared as follows: 15 g of the partially deactivated Seasorb 43 and 6 g of Celite 545 are slurried together in 50 ml of 95% EtOH in a 125-ml glass-stoppered Erlenmeyer flask by shaking the flask vigorously by hand for 5 minutes. The slurry is then spread immediately over five 8 × 8-inch glass plates. The plates

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

are air-dried 10 minutes and then dried for a minimum of 2 hours at 100°. The plates may be stored in a moisture-free desiccator until needed.

Chromatography. The dicarbonyl bis(2,4-dinitrophenylhydrazones) are spotted on the plate in ethyl acetate, and the plate is developed (in the direction of application of the slurry) in an equilibrated tank in one of the following solvent systems: acetone-benzene-MeOH (75:21.5:3.5); acetone-benzene-MeOH (75:23:2); acetone-benzene-MeOH (75:20:5); or acetone-benzene-MeOH (75:15:10), whichever system is found to be most suitable for the particular conditions employed (i.e., muffle temperature and lot of Seasorb 43) (see below). At the end of the development (about 40 minutes), the plate is removed and the spots are circled with a pencil as the solvent evaporates from the plate.

RESULTS AND DISCUSSION

Separation of the 3 classes investigated is shown in Fig. 1. As in the class separation of the monocarbonyl-2,4-dinitrophenylhydrazones, the

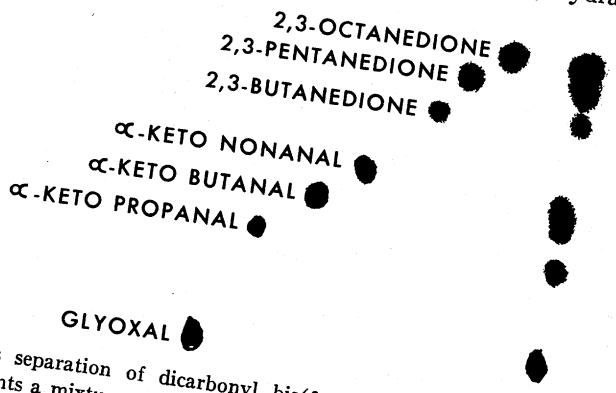

FIG. 1. Class separation of dicarbonyl bis(2,4-dinitrophenylhydrazones). Column on right represents a mixture of all members on the left.

lowest member of the class tends to lag behind the higher members. In the case of class separation of the monocarbonyls, the lower member moves into the class immediately behind. However, as shown in Fig. 1, with the dicarbonyls, the lower member does not go with the class immediately following; thus separation of the classes is cleanly effected and the slight lagging behind of diacetyl and methyl glyoxal may be beneficial in that it aids in their identification when either or both are present in a mixture. The 2,3-diketones are violet on the finished plate; the α-keto aldehydes

and glyoxal are blue. These colors were described by Schwartz (*3*) for the members of these classes when subjected to partition chromatography in ethanolamine-water-benzene systems. The color difference in the class separation is thus an additional aid to the final classification of an unknown. The 2,3-diketone class may appear yellow as the solvent ascends the plate but reverts to violet as the solvent evaporates from the completed chromatogram.

Six lots of Seasorb 43 were evaluated for their suitability as adsorbents for the class separation. At a fixed muffle furnace temperature and time (525°, 16 hours), and in a given solvent system, some variation in the adsorptive strength of the different lots was apparent. However, excellent separation of the classes with every lot could be obtained in one or more of the solvent systems given in the methods section. Moreover, it was also found that strict control of the muffle temperature was not essential if the heated lot was subject to the solvent-system screening to ascertain which system gives best separation of the classes. All of the compounds shown in Fig. 1 were chromatographed in the solvent-system screening of the various lots of magnesia. It is recommended that knowns be run simultaneously with an unknown or a mixture of unknowns. All of the diketone parent compounds are commercially available as is methyl glyoxal and glyoxal.

Sieving of the magnesia and Celite was found to be unnecessary. Solvent fronts ran perfectly straight, and, in fact, superior plates were obtained with unsieved as opposed to sieved material.

Prepared plates were deactivated rapidly when unduly exposed to moist air, so that one should work quickly once the plate is removed from the oven or the desiccator.

Very small amounts of the dicarbonyl bis(2,4-dinitrophenylhydrazones) can be visualized on the finished chromatogram due to the natural blue or violet color produced on magnesia. Approximately 0.01–0.02 μg of diacetyl bis(2,4-dinitrophenylhydrazone) (4×10^{-5} μmole), for example, can readily be seen. This amount corresponds to about 0.003 μg of the parent compound.

SUMMARY

A procedure is described for the separation of the bis(2,4-dinitrophenylhydrazones) of dicarbonyls into classes. The classes investigated were the 2,3-diketones, the α-keto aldehydes, and the dialdehyde, glyoxal. Separation was accomplished on weakened magnesia-Celite plates in an acetone-benzene-MeOH system in approximately 40 minutes. The method permits the classification of as little a 4 ×

10^{-5} μmole of a bis(2,4-dinitrophenylhydrazone). Diketone spots are violet; α-keto-aldehydes and glyoxal are blue.

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